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Review

Surface active drugs: self-association and interaction with membranes and surfactants. Physicochemical and biological aspects

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Abstract

Many pharmacologically active compounds are of amphiphilic (or hydrophobic) nature. As a result, they tend to self-associate and to interact with biological membranes. This review focuses on the self-aggregation properties of drugs, as well as on their interaction with membranes. It is seen that drug–membrane interactions are analogous to the interactions between membranes and classical detergents. Phenomena such as shape changes, vesiculation, membrane disruption, and solubilization have been observed. At the molecular level, these events seem to be modulated by lipid flip-flop and formation of non-bilayer phases. The modulation of physicochemical properties of drugs by self-association and membrane binding is discussed. Pathological consequences of drug–membrane interaction are described. The mechanisms of drug solubilization by surfactants are reviewed from the physicochemical point of view and in relation to drug carrying and absorption by the organism.

Keywords: Surface active drug; Drug self-association; Drug–membrane interaction; Drug–surfactant interaction; Membrane solubilization; Drug delivery

1. Introduction

Many pharmacologically active compounds are amphiphilic or hydrophobic molecules, which may undergo different kinds of association, and whose site of action in the organism frequently is the plasma membrane. Even if their target is intracellular, the interaction with this first barrier plays a fundamental role.

Amphiphilic compounds bear an ionic (zwitterionic, anionic or cationic) or non-ionic polar head group and a hydrophobic portion. In aqueous medium, they are able to organize themselves as micelles, bilayers, monolayers, hexagonal or cubic phases. The spatial separation between the polar and non-polar moieties, as well as the molecular

Abbreviations: AmB, amphotericin B; BS, bile salt; CAD, cationic amphiphilic drug; CD, circular dichroism; cmc, critical micellar concentration; CPZ, chlorpromazine; DBC, dibucaine; DOC, deoxycholate; DSC, differential scanning calorimetry; EPR, electron paramagnetic resonance; GI, gastrointestinal; HHB, hydrophilic–hydrophobic balance; LA, local anesthetic; Lyso PC, lysophosphatidylcholine; MM, mixed micelle; NMR, nuclear magnetic resonance; *P*, partition coefficient; PC, phosphatidylcholine; PEG, polyethylene glycol; PM, polymeric micelle; POE, polyoxyethylene; RBC, red blood cell; RES, reticuloendothelial system; SDS, sodium dodecyl sulfate; TFP, trifluoperazine; TI, therapeutic index; TTC, tetracaine

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shape [1] and the hydrophilic–hydrophobic balance (HHB) [2], determines their tendency to form the different structures, which eventually can be interconverted as a function of pH, temperature, ionic strength, concentration.

Surfactants (detergents) tend to associate as micelles; in these structures the hydrophobic portion is sequestered from the highly polar aqueous medium by a surrounding, approximately spherical, shell formed by the polar or ionic head groups. Classical surfactants have been extensively studied, both in terms of their self-aggregation properties and for their capacity to interact with model and biological membranes, promoting lysis, extraction of specific components (proteins or lipids), and ultimately, solubilization [3,4].

Micelle formation can be envisioned as a stepwise process, characterized by a series of equilibria and equilibrium constants or as a phase separation (all or none) process such that, once a critical concentration (the critical micellar concentration, cmc) is reached, further addition of the surfactant will result in aggregation [5]. Several authors have discussed the energetics of micelle formation [1,6–8]. Micelles are characterized by physicochemical parameters such as the cmc, aggregation number (N), particle size, HHB. This is a very simplified picture, since even for typical micelle-forming systems, pre-micellar aggregation has been described [9,10]. Moreover, micelles are polydisperse and the aggregation number represents the most probable one within a distribution [5]. Micellar shape is usually assumed to be spherical, but micelles are known to grow with increasing detergent concentration, becoming cylindrically shaped [5].

Micelles are highly dynamic structures, the equilibrium between monomer and aggregate being a fast process (lifetime of the order of microseconds). The dynamic nature of these aggregates is also reflected in the motional properties of the monomers within the micelle. They can rotate about their long molecular axes [9,10], diffuse laterally along the micellar surface [11], and, when formed by alkyl chain-containing surfactants, the chains, being highly flexible, can undergo segmental motion [9,10]. In comparison with other aggregates, such as bilayers, micelles are more loosely packed and less stable [12].

Because of their strong tendency to aggregate, alkyl chain-containing surfactants seem to associate according to the phase separation model. Normally, these micelles exhibit N between 50 and 200 [5]. Other detergents display a different aggregation behavior. A classical example is that of bile salts (BS), whose mechanism of micelle formation corresponds to a stepwise association [13]. N is usually much smaller than those of micelles formed by alkyl chain detergents. BS micelles are formed by juxtaposition of hydrophobic domains of adjacent molecules. The aggregation of BS is complex; for additional information, the reader is referred to the chapter by Krathovil in this issue and references therein.

Surface active drugs of quite different chemical structure are reported to self-associate and bind to membranes, causing disruption and solubilization, in a detergent-like manner.

Classes of amphiphilic drugs include phenothiazine [14–24] and benzodiazepine [25] tranquilizers, analgesics [26], peptide ([27] and references in Section 3.3) and non-peptide [28,29] antibiotics, tricyclic antidepressants [30–32], antihistamines [33], anticholinergics [34], β-blockers [35], local anesthetics (LA) [12,36–39], non-steroidal antiinflammatory drugs [40], anticancer drugs [41]. Many of these drugs contain one or more (condensed or not) aromatic nuclei, while others are of peptide nature. Table 1 lists the cmc and aggregation number (under specific conditions) of some commonly used non-peptide drugs. Fig. 1 presents structures of drugs that will be frequently referred to in this paper.

The literature on surface active drugs is vast and multifocused, rendering it a difficult task to elaborate a comprehensive review on the subject. In view of this, we will restrict the present article to examples focusing on compounds and topics that have been largely studied. A great deal of data on the surface active properties of drugs can be found in the book by Attwood and Florence [45], and other reviews [46–48].

We will focus on the surface active properties of drugs from the point of view of: (i) their self-aggregation properties; (ii) their interaction with membranes; (iii) the use of classical surfactants as solubilizers of amphiphilic and hydrophobic drugs and their use in drug delivery. Some shorter topics (see Sections 7–9) will also be discussed.

2. Self-association of amphiphilic drugs

The aggregation of surface active drugs follows the same principles as classical detergents. While some drugs display the ability to self-associate forming closed, micelle-like structures, others aggregate by continuous stacking [48]. When micelles are formed, their aggregation number usually is small (Table 1). Drug self-association is also temperature-, ionic strength-, and pH-dependent. The cmc of a series of piperazine-containing drugs increased and N decreased with decreasing pH, due to protonation of

Table 1
Micellar properties of some non-peptide surface active drugs in water

Class	Drug	cmc (M)	N	Ref.
Analgesics	Dextropropoxyphene	1.0×10^{-1}	7	[45]
Antibiotics	Actinomycin D	1.0×10^{-4}	–	[45]
	Penicillin G	2.5×10^{-1}	–	[45]
	Streptomycin	9.0×10^{-5}	–	[45]
	Sodium fusidate	3.6×10^{-3}	–	[44]
Anticholinergics	Adiphenine·HCl	8.2×10^{-2}	10	[45]
	Chlorphenoxamine·HCl	4.5×10^{-2}	13	[45]
	Orphenadrine·HCl	9.6×10^{-2}	7	[45]
	Penthianate methobromide	2.2×10^{-1}	6	[33]
	Piperidolate·HCl	8.2×10^{-2}	12	[45]
Antifungal polyenes	Amphotericin B	6.0×10^{-7}	–	[240]
	Nystatin	3.0×10^{-6}	–	[42]
Antihistamines	Bromodiphenylhydramine·HCl	5.4×10^{-2}	11	[33]
	Chlorcyclizine·HCl	1.27×10^{-1}	3	[45]
	Diphenhydramine·HCl	9.0×10^{-2}	9	[33,45]
	Diphenylpyraline·HCl	4.0×10^{-2}	9	[33,45]
	Thenyldiamine·HCl	1.0×10^{-1}	3	[45]
	Tripelennamine·HCl	1.2×10^{-1}	3	[45]
Antihypertensives (with β-blocking action)	Acetobutolol·HCl	1.7×10^{-1}	4	[45]
	Oxprenolol·HCl	1.7×10^{-1}	3	[45]
	Propranolol·HCl	1.0×10^{-1}	12	[33,45]
General anesthetics	Thiopental	7.0×10^{-3}	–	[62]
Local anesthetics	Dibucaine·HCl	6.6×10^{-2}	–	[104]
		1.1×10^{-2}	9	[37]
	Stadacaine·HCl	7.6×10^{-2}	17	[45]
	Tetracaine·HCl	1.3×10^{-1}	7	[39,43]
		6.0×10^{-2}	–	[12,36]
Phenothiazines	Chlorpromazine·HCl	1.9×10^{-2}	11	[45]
		2.2×10^{-2}	12	[18]
	Promazine·HCl	3.6×10^{-2}	11	[45]
		1.2×10^{-2}	–	[16]
	Promethazine·HCl	4.4×10^{-2}	9	[45]
		5.8×10^{-2}	12	[18]
	Thioridazine·HCl	5.9×10^{-3}	8	[45]
		1.5×10^{-3}	–	[16]
	Trifluoperazine	4.2×10^{-5}	–	[104]
	Trifluopromazine	4.5×10^{-3}	–	[16]
Thioxanthene tranquilizers	Flupenthixol	8.5×10^{-3}	19	[45]
Tricyclic antidepressants	Amitriptyline·HCl	3.6×10^{-2}	7	[45]
	Butriptyline·HCl	4.2×10^{-2}	9	[45]
	Clomipramine·HCl	2.2×10^{-2}	6	[45]
	Desipramine·HCl	4.9×10^{-2}	7	[45,62]
	Imipramine·HCl	4.7×10^{-2}	8	[18,45]
	Nortriptyline·HCl	2.3×10^{-2}	4	[45]

the second nitrogen atom of the drugs' piperazine ring [49].

We will describe below some properties of two well studied families of drugs, the phenothiazine tranquilizers and the polyene antibiotics.

2.1. Phenothiazine tranquilizers

Phenothiazines aggregate in a micelle-like manner, N being of the order of 6–15 [14–24]. The drugs were seen to form pre-micellar aggregates [23]. The phenothiazine ring system (Fig. 1, chlorpromazine) is V-shaped about an axis from the N to S atoms with an internal angle of about 155° [50]. Nuclear magnetic resonance (NMR) data suggest a concave-to-convex stacking of molecules within the pre-micellar and micellar aggregates, with the alkyl side chains on alternate sides of the stacks [16,51]. It was proposed that micelles are formed by several short stacks hydrophobically bonded together, generating roughly symmetrical systems [16]. This mechanism would allow for the formation of higher aggregates and would explain the larger values of N calculated for drugs from this family [52,53].

The cmc of phenothiazine micelles decreases and N increases with increasing electrolyte concentration. At higher salt concentrations, a spherical-to-rod transition occurs. The cmc decreased and N increased in the order $F^- < Cl^- < Br^- < I^-$ [16]. At higher I^- concentrations and in the presence of phosphate, phase separation was observed. The order of the effects correlated with the position of the ions in the Hofmeister series. The effects were discussed in terms of an interplay of factors including the affinity of the counterion for the aggregate, counterion hydration energy, and ion effects on water structure.

The cmc of chlorpromazine (CPZ) is pH-sensitive, increasing with decreasing pH. Above the cmc, CPZ micelles undergo a concentration-, temperature-, and pH-dependent transition leading to phase separation, which is followed by a sudden increase in light scattering. Spin label electron paramagnetic resonance (EPR) spectra indicated an increase in intramicellar compactness at higher pH, due to a decrease in head group repulsion [17].

Aggregation also affects the apparent pK (pK_{app}) of ionizable compounds. The pK_{app} of trifluoperazine (TFP) decreased with increasing drug concentration [49]. pK shifts are also observed upon binding of drugs containing ionizable groups to bilayers or micelles (see Section 4.1).

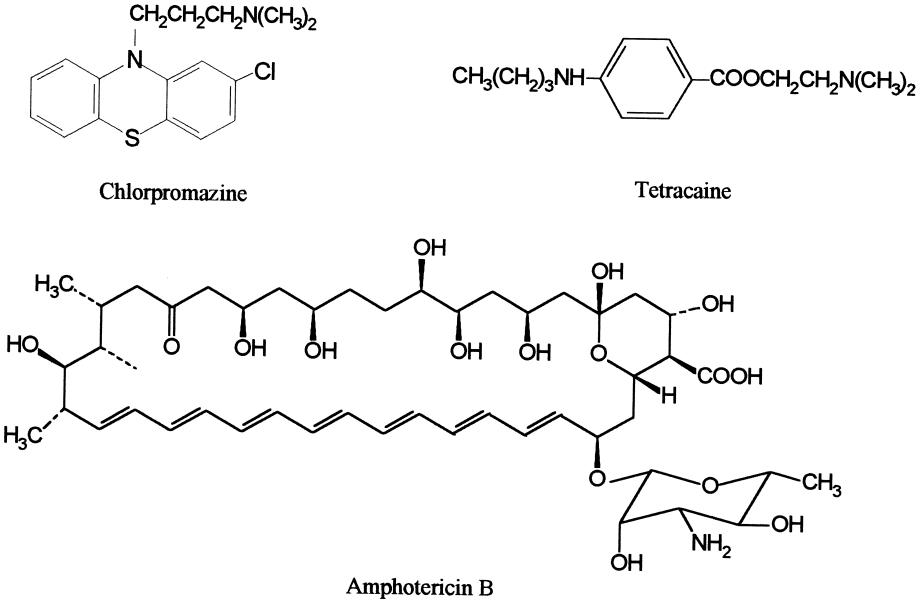


Fig. 1. Structures of some surface active drugs discussed in this paper.

2.2. Polyene antibiotics

Self-association also occurs in another class of drugs, the polyene antibiotics, used against mycotic infections. One member of this family, amphotericin B (AmB), is widely used in the treatment of systemic infections (to which immunodepressed patients are very sensitive), in spite of its toxic effects, the most serious being nephrotoxicity. Because of its clinical importance, AmB has been one of the first drugs used in patients in liposomal form, which greatly decreases its toxicity.

AmB (Fig. 1) is highly water-insoluble ($\sim 10^{-7}$ M) and its aggregation behavior is extremely complex. The toxic and therapeutic effects of AmB were seen to correlate with the particle size of the intravenously injected antibiotic [54], implying that these effects are modulated by its aggregation state. The long time used clinical preparation (Fungizone) consists of a 1:2 (mol:mol) AmB:deoxycholate (DOC) complex. The circular dichroism (CD) spectra of this preparation in aqueous ethanolic solution suggested that AmB forms helical aggregates [55]. The aggregation properties of Fungizone were examined by spin labeling [56] and by quasielastic light scattering [57]. These studies indicated that Fungizone consists of large aggregates rather than small mixed micelles (MM), as is usually implied in the literature, and that the system is quite unstable, ultimately decomposing into solid AmB and DOC micelles. The kinetics is faster in the presence of salt. At high concentration, the mixed aggregates coexist with pure DOC micelles that serve as a pool of DOC, and stabilize the mixed aggregate. Dilution below DOC's cmc leads to a faster loss of aggregate stability. The particle size increased with decreasing AmB-DOC concentration, probably due to loss of DOC from the aggregates.

In aqueous solution the drug has been found to exist as a mixture of various species: monomers and soluble, as well as insoluble, aggregates, whose concentration depends on factors such as total antibiotic concentration, method of preparation, and even the concentration of the stock solution [58]. In addition, temperature has pronounced effects: heating to 50–60°C causes formation of 'super-aggregates' [59]. The aggregation state of AmB also modulates the kinetics of its autoxidation [59–61].

2.3. Surface activity and drug penetration through the blood–brain barrier

Recently Anna Seelig and coworkers have devised a method to predict the ability of drugs to cross the blood–brain barrier (BBB) based on the drug's surface active properties [62,63]. The surface activity of drugs was quantified by their Gibbs adsorption isotherms in terms of three parameters: the onset of surface activity, the cmc, and the surface area requirement of the drug at the air–water interface. By plotting the cmc as a function of the concentration required for the onset of surface activity (C_o), three regions could be identified: the low cmc, low C_o region corresponded to very hydrophobic drugs, unable to cross the BBB because of their strong binding to the membrane; the intermediate region encompassed less hydrophobic drugs, that can diffuse through the BBB; the drugs in the third region are relatively hydrophilic and only cross the BBB when applied at high concentrations. Similar results were obtained in plots of cmc vs. $1/K_{aw}$, K_{aw} being the drug's air–water partition coefficient, a parameter that correlated with C_o. Over 50 drugs were analyzed according to these criteria and their location in these plots was in agreement with their ability to cross the BBB.

3. Surface activity effects in amphiphilic drug–membrane interactions

Amphiphilic drugs interact with membranes, model and biological, causing a variety of effects. The literature in this field is very large. Considerable advances in the understanding of the effect of drugs on the structural and dynamical properties of membrane components have been achieved through the use of spectroscopic (EPR, NMR, fluorescence, Fourier transform infrared, CD) and other biophysical (X-ray and neutron diffraction, differential scanning calorimetry (DSC), titration calorimetry, monolayers) techniques. We will not discuss here the effects of drugs on the degree of order and mobility of membrane lipids. Rather, we will focus on phenomena that can lead to membrane disruption and even solubilization, and that often are responsible for the drugs' toxic effects. Literature will be presented on

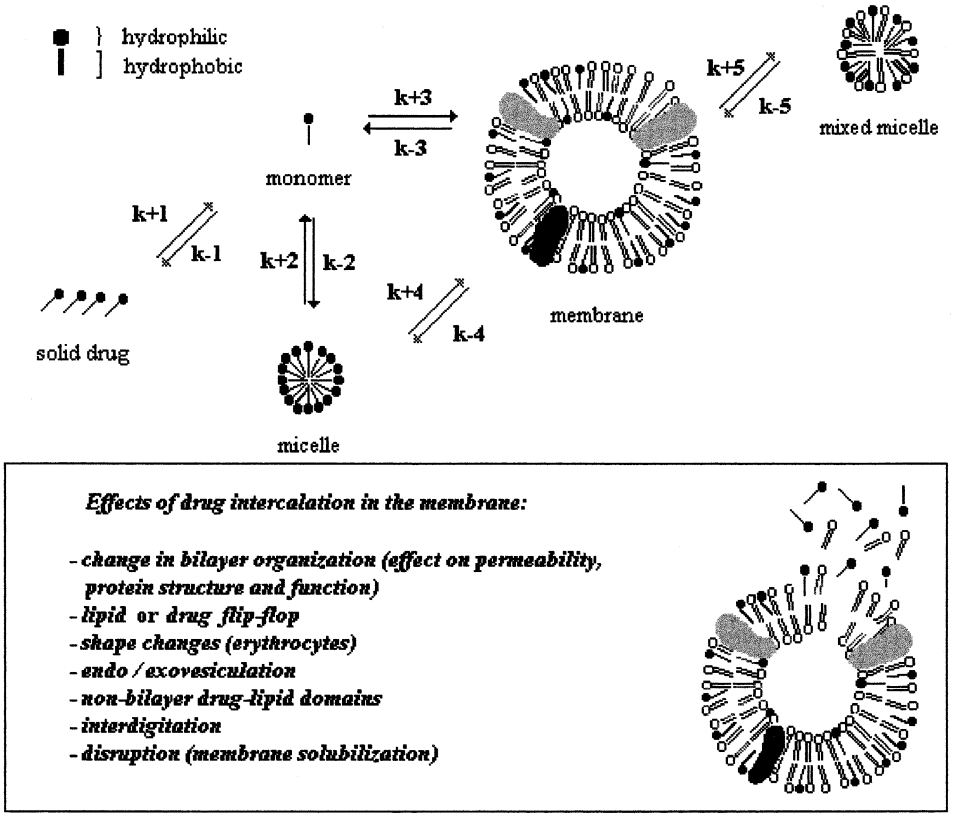


Fig. 2. Scheme of possible events taking place with an amphiphilic surface active drug starting from the solid. The processes corresponding to the equilibria are: 1 – solubilization; 2 – monomer–aggregate equilibrium; 3 – monomer binding to the membrane; 4 – aggregated drug binding to the membrane; 5 – membrane solubilization. Upon saturation drug molecules leave the membrane carrying membrane components, lipids and proteins. The *k*s represent rate constants. The possible events resulting from drug–membrane interactions prior to solubilization are listed in the scheme.

phenomena such as drug-induced lipid flip-flop, interdigitation, and formation of non-lamellar phases that could be related, at the molecular level, to the mechanism of the disruptive (surfactant) effects of drugs,

A theoretical analysis of the effects of drugs on lipid bilayers has been provided by Mouritsen et al. [64,65]. Assuming that drugs insert into membranes as interstitial components, it was shown that they affect the organization and thermotropic properties of the lipids. Computer simulations indicated that partitioning drugs accumulate heterogeneously in the membrane, higher concentrations being attained at the interface between gel phase and liquid crystal domains. As a consequence, local concentrations may be much higher than in the bulk aqueous phase or even in other regions in the lipid bilayer. This possibility should be borne in mind when discussing drug effects on membranes, especially in the context of the present article, where surface active properties of drugs are in focus. The microscopic description of drug insertion into bilayers provided by the work of Mouritsen and colleagues suggests that bilayer disruption by drugs could be modulated by micelle-like aggregates or other non-bilayer phases whose formation is induced by a local increased concentration.

Early work [46] attempting to evaluate the surface activity of drugs made use of their interaction with lipid monolayers at an air–water interface. Some pioneering studies along these lines have been performed by Skou [66] on LA. A classical review by Seeman [67] describes his and other studies on the membrane action of pharmacologically active compounds.

The conditions favoring membrane disruption usually imply a high drug:lipid molar ratio. This does not imply that such concentrations are not achieved in the organism since it has been shown that the liver/plasma ratios of tricyclic antidepressants are of

the order of 20–100:1 [68]. At high drug:lipid ratios (global, or as discussed above, local) the surface active properties of the drug come into play and one would expect processes similar to those operating with classical detergents. In the latter case, removal of membrane components occurs after the membrane becomes saturated and the detergent concentration in the aqueous phase reaches the cmc [69,70]. Further addition leads to removal of membrane components, giving rise to lipid–detergent, protein–detergent, or lipid–protein–detergent MM. Fig. 2 depicts the possible steps involved in this mechanism. A model providing a qualitative and quantitative analysis of this process was developed by Lichtenberg [69], and has been applied by Goñi and coworkers to the action of several detergents and detergent-like molecules [71].

In many studies of drug-induced membrane disruption, the state of the drug in the aqueous phase has not been examined. Nevertheless, since this process usually takes place at high drug:lipid ratios, it is conceivable that the drug would be aggregated in the aqueous phase.

3.1. *Drug-induced membrane phenomena: shape changes, lipid flip-flop, non-bilayer phase formation, vesiculation, solubilization*

A very commonly used model for studies of amphiphilic drugs is the red blood cell (RBC). Under hypotonic conditions, many drugs display a biphasic behavior in RBC: while low concentrations protect the membrane, higher concentrations produce hemolysis [67], probably by the mechanism depicted in Fig. 2. This biphasic behavior was also observed with classical surfactants [72,73]. The hypotonic hemolytic test has become a classical tool for studying drug–membrane interactions. Drugs exhibiting biphasic behavior are: LA, tranquilizers, antihistamines, antiinflammatory drugs, antiarrhythmics, sedatives, narcotics, polyene antibiotics, vitamin A, steroids, peptides [67]. At the onset of the lytic phase, in general, initially the permeability of small solutes and ions, like K^+, increases. As a result of the colloid–osmotic imbalance, other solutes permeate and, ultimately, large holes are formed causing the loss of cell contents, including proteins.

In the early 1970s, evidence started to accumulate for the asymmetric distribution of components in the outer and inner monolayers of biological membranes [74]. It was also known that drugs caused shape changes when added to erythrocytes. Two main types of changes occurred: the biconcave disk-shaped cells were converted to a crenated form (echinocytes) or to an invaginated or cupped form (stomatocytes). Moreover, Deuticke [75] had observed that most crenation inducers were anionic (free fatty acids, barbiturates), while cup formers were essentially cationic (phenothiazines, LA). Based on these observations, Sheetz and Singer [76] formulated their bilayer couple hypothesis, i.e. they suggested that the two halves of the membrane could respond differently to perturbations while remaining coupled to each other. It was suggested that if expansion of one monolayer in the plane of the membrane occurred with respect to the other, the two layers would still remain in van der Waals contact with each other, leading to various functional consequences, including cell shape changes. The authors proposed that cup formers would distribute preferentially into the cytoplasmic half of the bilayer, causing it to expand, and leading to invagination. Crenators, on the other hand, would intercalate preferentially in the outer monolayer, expanding this region and inducing echinocytosis. This behavior would be driven by the higher concentration of negatively charged phospholipids in the inner monolayer. At higher concentrations, both types of compounds caused the RBC to become spherical and lyse.

These ideas were tested by examining the interaction between RBC and drugs carrying an ionizable tertiary amine, as well as their quaternary ammonium counterparts. Scanning electron micrographs showed that, while the quaternary ammonium ion methochlorpromazine acted as a crenator, CPZ acted as a cup former [76].

The bilayer couple hypothesis exerted great influence and many authors have interpreted changes in membrane shape in terms of preferential location of incorporated amphiphilic solutes. A theoretical verification of the bilayer couple hypothesis was presented by Iglic and coworkers [77].

With the discovery by the group of Devaux [78] that lipid flip-flop rates in biological membranes are much faster than in model systems, studies were undertaken to verify whether drug-induced RBC shape changes were related to changes in lipid distri-

bution across the two halves of the membrane. Rosso et al. [79] and Schrier et al. [80] demonstrated that both vinblastine and CPZ caused time and concentration-dependent RBC stomatocytosis, as well as lipid scrambling. Later, Chen and Huestis [81] proposed that, while CPZ induces lipid redistribution over extended time periods, which may mediate the complex morphological transformations observed, the early stomatocytic response elicited by addition of CPZ is not due to lipid reorganization.

Another phenomenon known to destabilize the bilayer structure is the formation of non-lamellar phases. The bilayer to hexagonal II (HII) phase transition has been implied in the mechanism of membrane fusion [82] and is known to depend on lipid nature, shape, temperature, and on the presence of additives, such as Ca^{2+} ions, and drugs. The local formation of non-lamellar (HII) phases in a membrane could give rise to a region that would destabilize the bilayer, creating an eventual site for the initiation of cell disruption. In work dealing with several kinds of amphiphiles, Isomaa et al. [83] suggested that the observed shape changes would be due to the formation of non-bilayer intrabilayer phases. In addition, transmembrane lipid redistribution was also observed. Indeed, the induction of HII phase formation by the peptide antibiotic gramicidin A in RBC was seen to occur under the same conditions that favored lipid flip-flop [84].

Lipid flip-flop in RBC membranes was also induced by AmB [85], LA [85], gramicidin A [86], aliphatic [87] and aromatic [88,89] alcohols. Flip-flop of an anticancer drug, doxorubicin, was also shown to occur across erythrocytes, as well as lipid membranes [90].

Several drugs were found to influence the bilayer to HII phase transition in model, as well as biological membranes. CPZ [91,92], tetracaine (TTC) [92], and dibucaine (DBC) [91–93], as well as the anticancer drugs adriamycin [94] and doxorubicin [95] have been found to favor [93,95] or to prevent [91,92,94] the bilayer to HII phase transition, depending on the lipid composition.

Drugs, similarly to classical surfactants, also induce exo- and/or endovesiculation from RBC ([96], Malheiros, Brito, Brites and Meirelles, submitted). Sphero-echinocytogenic amphiphiles induce exovesiculation, whereas stomatocytogenic amphiphiles induce endovesiculation. Since vesiculation implies a fusion event, again the likelihood of non-bilayer phase formation has to be considered. The involvement of specific states of lipid asymmetry was also suggested. Vesicular fragments from RBC membranes treated with amphiphilic drugs at concentrations lower than lytic were extracted by hygroscopic desorption filtration [97]. This phenomenon was suggested to be due to amphipath-induced gross redistribution of components in the plane of the membrane.

One of the most studied amphipathic drugs, CPZ, was also shown, by freeze fracture electron microscopy, to induce intramembrane particles clustering and formation of round patches nearly devoid of these particles [98], to inhibit RBC cholinesterase by a micellar mechanism [99], and to solubilize RBC [100] and synaptic membrane proteins [101].

Yasuhara et al. [102] suggested that the study of membrane effects of drugs on erythrocytes could be useful for screening for hepatotoxicity in vitro.

Although it is widely believed that drugs seem to cause cell lysis by a mechanism very similar to that described for classical detergents, not many studies have been performed with the scope of demonstrating this sequence of events. Malheiros et al. studied the interaction of the antipsychotic drug TFP and the LA DBC with RBC. Both TFP and DBC display the biphasic effect [103,104]. The authors found that at pH 7.4, when the drug is mainly positively charged, TFP micelles are formed. Above its cmc (Table 1), TFP induced membrane disruption and release of drug-bound membrane lipids into the medium (Malheiros, Brito, Brites and Meirelles, submitted). In contrast, for DBC, drug binding increased until membrane saturation was reached, but hemolysis occurred at an anesthetic concentration below the cmc.

Disruption and solubilizing effects of drugs upon model membranes have been reported. It has been shown that the cationic form of the LA TTC forms micelles [12,36] that destroy the bilayer structure of egg phosphatidylcholine (PC) vesicles, giving rise to mixed TTC–PC micelles, as seen by light scattering [36] and spin probes EPR spectra [12]. Dilution to concentrations below the anesthetic's cmc restored the bilayer structure. These observations are in line with reported toxic effects of the LA [105,106].

CPZ was found to solubilize lipid membranes (via MM formation) [107,108] and to promote endovesiculation [109]. Endovesiculation was also promoted by DBC and by safingol [109]. Liposome solubilization was triggered by the weakly ionizable drugs propranolol [110] and cefotaxime [111]. The non-steroid antiinflammatory drugs diclofenac [112] and ibuprofen [113] converted liposomal dispersions into drug-containing MM. Piracetam, a non-ionizable, non-metabolized drug that improves erythrocyte deformability, caused changes in the ^{31}P NMR spectra of PC membranes that were ascribed to an isotropic phase due to mobile drug–phospholipid complexes [114]. Garcia and Perillo [115] found that flunitrazepam caused a decrease in aggregation number and stability, and an increase in the curvature of dipalmitoyl PC vesicles. Negative staining electron microscopy showed a decrease of 2–3 times of the mean vesicle diameter.

3.2. AmB–membrane interactions

The binding of AmB to biological and model membranes has been studied for a long time. Its mechanism of action is thought to involve interaction with sterols, leading to pore formation and increased permeability, and, ultimately, to membrane disruption and cell lysis (however, see [116]). The AmB pore was proposed to consist of antibiotic molecules intercalated with sterol molecules [117,118]. Single channel measurements suggested a dynamic nature for the pore [119]. The higher affinity for fungal ergosterol over mammalian cholesterol has been invoked as the explanation for the antibiotic's selectivity for the fungal membrane. AmB is able to lyse RBC and to induce lipid flip-flop [85]. In model systems, it can cause a very fast loss of the inner contents of egg PC–cholesterol vesicles [120], possibly through a detergent-like effect [121].

Since the equilibrium between monomers and aggregates appears to play a key role in drug activity, in more recent years, work has aimed at understanding the membrane effects of the antibiotic in terms of its aggregation state and of the differences in its affinities for cholesterol and ergosterol as a function of the aggregation state. Bolard et al. [122] found that the binding of the antibiotic to membranes in monomeric or in aggregated form depends on the lipid composition, and toxic effects were ascribed to the aggregated state. Thus, while aggregated AmB is capable of lysing both RBC (cholesterol-containing membranes) and fungi (ergosterol-containing membranes), the monomer is active only against the latter. We will return to this topic, when discussing the use of surfactants for drug delivery (Section 6.3).

The effect of AmB aggregation state on its interaction with ergosterol- and cholesterol-containing phospholipid monolayers was investigated at antibiotic concentrations where it existed either as monomers, or as micelles, or as aggregates of micelles [123]. While the monomer interacted only with ergosterol-containing monolayers, the micellar form interacted with monolayers containing either sterol, in a differential manner. It was proposed that the activity of AmB is most likely related to its micellar form. The large micellar aggregates were able to extract cholesterol from the monolayer, and were suggested to be the antibiotic's toxic form.

DSC studies not only corroborated the previously observed differences between cholesterol- and ergosterol-containing membranes [124] but also indicated that AmB is able to interact with pure dipalmitoyl PC, as previously seen by CD spectroscopy [125] and by permeability enhancement [120]. Phospholipid complexation by AmB in chloroform solution was also demonstrated by means of CD and NMR [126].

Studies with sterols, in aqueous or mixed aqueous–organic solvents, paralleled the results obtained with model membranes or in toxicity studies, indicating that AmB interacts more favorably with ergosterol than with cholesterol [127–129].

3.3. Antimicrobial peptides

Many peptides possess pharmacological activity. A great number are active against bacteria, fungi, viruses, and, sometimes, tumors. They are synthesized by all forms of life, from bacteria to vertebrates, including mammals, and also by plants (for reviews see [27,130]). Indeed, at present, the notion exists that a parallel non-immune defense system exists based on the ability of organisms to synthesize peptide antibiotics. The research in this field is very intense, especially in view of the need of new drugs because of bacterial resistance to the presently used antibiotics. This intense activity can be appreciated

by the publication of two recent review issues (Biopolymers, Volume 47, number 6, 1998, and Biochim. Biophys. Acta, Volume 1462, number 1, 1999), in addition to some excellent review articles on this topic [27,130–136].

Various antibiotic peptides possess D- (gramicidin A) or unusual amino acids (alamethicin). Some are bound to a fatty acid chain at the N-terminal; in some cases the acyl chain carries a hydroxyl group that is esterified by the peptide C-terminal, forming a cycle (polymyxin B). Interestingly, some peptides carry a great number of residues of the same amino acid (histatin, from human saliva, rich in histidine). Most antimicrobial peptides are amphipathic, highly positively charged molecules. This has served as the basis for the contention that their target site and their selectivity with respect to bacteria (and tumor cells) is the cell membrane [27,130], since in these systems negatively charged phospholipids are present in the outer monolayer to a larger extent than in mammalian host cells (however, see [137]). The fact that all-D amino acid-containing peptides retain essentially the same activity as their native counterparts has been taken as an indication for the absence of specific membrane receptors for these peptides [138].

From the conformational point of view, they can organize themselves as linear (magainin, pardaxin, cecropin, dermaseptin) or cyclic (alamethicin) α-helices, or β-sheets. β-Sheet-forming peptides are cyclized by one (brevinin-1) or more (protegrin I, tachyplesin I, β-defensin-1) disulfide bonds or by lactone formation (gramicidin S, tyrocidin) [130]. The spatial organization of gramicidin S is such that, while the hydrophobic residues are on one side of the backbone, the polar and ionic residues point in the other direction [139]. The extensively studied antibiotic gramicidin A forms a β-helix [140]. In general, the peptides have a random conformation in dilute aqueous solution and acquire secondary structure upon binding to membranes (nevertheless, see below).

Peptide antibiotics act by increasing membrane permeability. The molecular mechanism(s) by which these peptides increase membrane permeability to ions is the subject of an ongoing discussion. Gramicidin A and alamethicin ion channels have been extensively studied. The channel formed by gramicidin A β-helix allows water and ion passage [140]. It is widely accepted that alamethicin acts according to the 'barrel-stave' model (see below).

Several models have been proposed or shown to operate for linear α-helical peptides. The 'barrel-stave model' (Fig. 3B) consists of a bundle of peptide molecules inserted in the bilayer in an amphipathic α-helical conformation with their axes perpendicular to the surface and their polar and charged amino acid residues lining the interior of an aqueous pore. Nevertheless, while this model has been adequate for certain peptides (alamethicin, pardaxin), it clearly is not suitable for others (magainin, dermaseptin). Moreover, some peptides were found to be aligned with the helix axis parallel to the bilayer normal [135], leading to the proposal of several mechanisms that take this feature into account. One of these, the 'carpet model' [132], is depicted in Fig. 3A. According to this model, the peptides initially bind to the membrane surface in a carpet-like manner. Membrane permeation would occur at high peptide concentration and disruption would take place when the whole surface is covered by a monolayer of peptides. Other models have been proposed whose initial steps imply peptide binding to the membrane surface: the toroidal model, by Matsuzaki [133], the wormhole model, by Huang [141], the in-plane diffusion model, by Bechinger [135]. It has been pointed out that all models have features in common [134,135]. They all imply a detergent-like effect, with peptide–lipid particles exiting the membrane.

Experimentally, antimicrobial peptides have been shown to aggregate in the membrane [142,143], as well as in aqueous [142,144–146] or aqueous–organic phase [147]. As for non-peptide drugs (see Section 3.2), hexagonal phase formation (gramicidin A) [84] and induction of lipid flip-flop (gramicidin A [86] and magainin [133]) have been observed. Alamethicin [148] and gramicidin S [149] were shown to induce cubic phase formation and the strongly lytic peptide polymyxin B was seen to cause lipid interdigitation [150]. Gramicidin S was reported to stimulate the release of phospholipids from RBC and shape changes (spiculation) were seen to occur prior to the release of membrane components [151]. It was proposed that the peptide molecules accumulated in the outer half of the bilayer, causing crenation, rendering the membrane structure unstable, with a con-

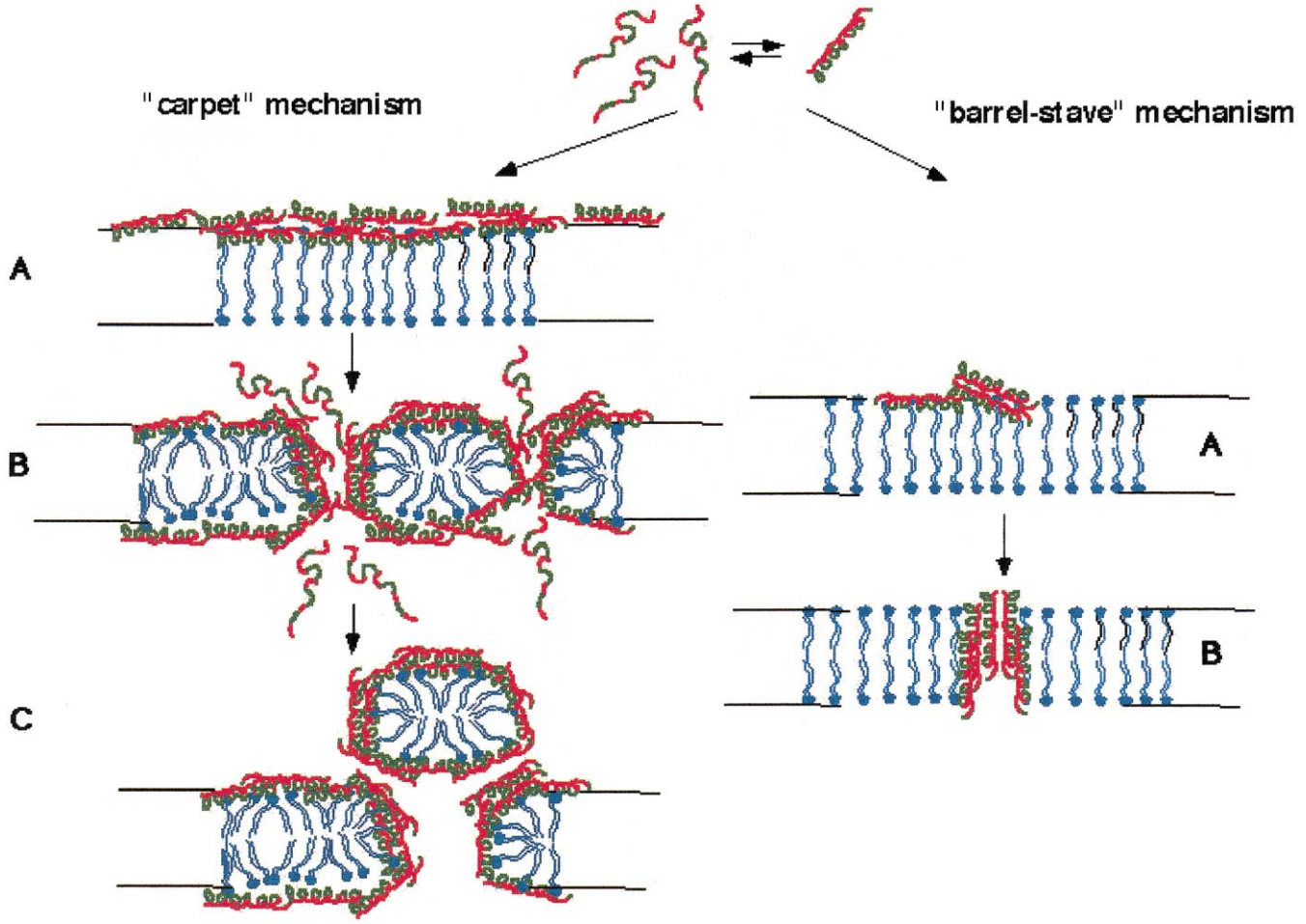


Fig. 3. Possible mechanisms for peptide–membrane interaction. (A) The carpet-like mechanism, whereby peptide molecules line up on the membrane surface until a critical concentration is reached and a detergent-like effect takes place. At this stage, peptide and membrane components form aggregates that leave the membrane, causing disruption. (B) The barrel-stave mechanism, whereby a peptide bundle spans the lipid bilayer giving rise to pores that promote increased permeability and, eventually, membrane lysis, by a colloid osmotic mechanism. It should be noted that at high enough concentration, aggregation in the membrane has been found also in the case of peptides that function through the pore mechanism, also leading to membrane disruption (see text). Reproduced, by permission, from [132].

sequent release of membrane components. The peptide's mechanism of action was compared to that of classical surfactants. The detergent-like effect of peptide antibiotics has been widely documented [132,135]. In fact, some cannot be used for patients because of their membrane-disrupting effects.

3.4. *Measurement of binding constants and partition coefficients*

The analysis of the effects of drugs upon membranes requires the knowledge of the actual amount of bound drug. The binding of solutes to aggregated systems has been analyzed in terms of either binding or partitioning formalisms.

K_b, the binding constant, is given by:

$$K_b = \frac{[SM]}{[S][M]} \quad (1)$$

where [S] and [SM] are the concentrations of free and bound solute, respectively, and [M] is the concentration of the aggregate-forming compound.

The partition coefficient, P, can be defined as:

$$P = \frac{n_m / V_m}{n_w / V_w} \quad (2)$$

where n corresponds to the number of moles of solute, V to volume, and the subscripts m and w to the membrane (or micellar) and water phases, respectively.

The K_b formalism implies that the water solubility is not a limiting factor and that saturation is reached due to the existence of saturable binding sites, yielding a hyperbolic binding isotherm. The partition coefficient formalism coincides with the linear part of the binding isotherm. In this region, the partition coefficient and K_b are related by $K_b = P.\bar{V}$, where $\bar{V}$ represents the lipid partial molar volume [152].

Nevertheless, a large number of drugs have limited water solubility, which determines their binding to an aggregate, i.e. transfer to the aggregate ceases when the aqueous solubility is reached, and the aqueous phase becomes saturated (see below).

When working with biological or model membranes, the most common procedure to determine P is centrifugation and measurement of the solute fraction remaining in the aqueous phase. This procedure has disadvantages, such as the need to separate both phases, the centrifugation time (and eventual chemical reactions taking place during this time), and the risk of some membrane remaining in the aqueous phase, and/or some poorly soluble solute co-sedimenting without actual partitioning [153].

Our laboratory has been engaged in the development of methodologies to determine partition coefficients without the need of phase separation by making use of spectroscopic techniques such as EPR, NMR, fluorescence. The basic principle consists in measuring an effect proportional to the amount of bound drug. A set of equations can be derived for the calculation of P [154]. A comparison between P values obtained from fluorescence, spin labeling and centrifugation data showed good agreement [154–156].

Another method [155] is based on the knowledge of the aqueous solubility of the partitioning compound and on the assumption of the following equilibrium:

$$\text{Solid} \rightleftharpoons \text{Solute in solution} \rightleftharpoons \text{Membrane-bound solute}$$

A solute distributes between water and membrane until its water solubility (S_w, saturation) is reached. When this happens, no more solute will partition into the membrane, further addition leading to precipitation of the solid. At this point, a solute-dependent membrane property will reach a maximum. Since n_w is known (from S_w), so is n_m ($n_m = n_{total} - n_w$), allowing the calculation of P (Eq. 2). P values for a series of LA determined by this procedure, using fluorescence and EPR measurements, were in very good agreement with those obtained by centrifugation [155].

Fluorescence measurements have been employed to determine binding constants of several drugs to membranes or micelles [157–161].

3.5. *Effect of water structure on binding of drugs to membranes and micelles*

Ions from the Hofmeister series and agents such as urea are known to affect the solubility of relatively non-polar compounds in water, as well as their distribution between water and organic solvents (for a review, see [162] and references therein). This is mainly due to the effect of the additives on water structure. Kosmotropes (sulfate, phosphate, chloride) increase water order and decrease solute solubility, while chaotropes (bromide, thiocyanate, perchlorate) do the opposite. Ions from the Hofmeister series alter properties of micelles such as the cmc and the aggregation number In addition, chaotropic anions bind to lipid membranes with high affinity [163].

We have examined the effect of anions of the Hofmeister series and urea, on the partitioning of charged and uncharged TTC into zwitterionic micelles [161] and phospholipid bilayers (Sakabe, Casallanovo, Teixeira and Schreier, in preparation). The binding of both forms was affected, varying in the order: $ClO_4^- < SCN^- <$ urea $<$ buffer $< Cl^- < SO_4^{2-}$. The ions also caused changes in micellar packing and size. The results were analyzed in terms of the effects on water structure, ion binding, and changes in micellar organization, with prevalence of the former.

4. Effects of aggregation on physicochemical properties of drugs

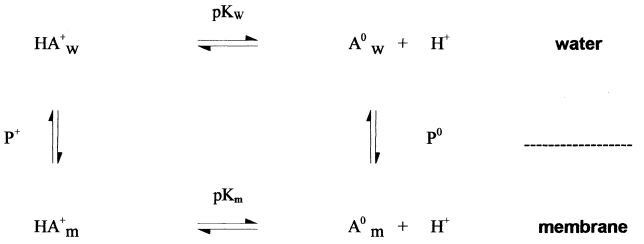
Amphiphile aggregation gives rise to regions of different physicochemical properties. Thus, while

the hydrophobic core provides an environment of very low polarity, the head group region consists of a shell with high charge concentration, in the case of ionic surfactants, or, at least, of higher polarity, in the case of non-ionic surfactants. As a result, the incorporation of an amphiphilic drug into an aggregate, either upon self-association or by intercalation into other organized assemblies (bilayers, emulsions, or micelles) will affect its physicochemical properties, e.g. the degree of ionization, reaction rates, and, eventually, reaction mechanisms.

4.1. Charge effects: pK shifts

In 1976, McLaughlin and Harary published an analysis of the binding of charged species to lipid bilayers based on the Guy–Chapman theory [164]. This work was extended to ionizable compounds by Lee [157], who showed that whenever the binding constants for the charged and uncharged forms are different, this will imply a pK shift. The binding constant for the charged species was described as consisting of an intrinsic value plus the contribution of charge effects due to the ligand itself, of the ions in solution, and the charge on the lipid head group [157,165,166].

Several definitions of pK for compounds partitioning between membrane and aqueous phase have been presented [167]. We have defined an apparent pK, pK_{app}, that corresponds to the pH in the aqueous phase where the sum (charged species in the membrane+charged species in the aqueous phase) equals the sum (uncharged species in the membrane+uncharged species in the aqueous phase) [168]. It should be noticed that pK_{app} will depend on membrane concentration. Therefore, if one of the forms, charged or uncharged, of a drug is the pharmacologically active species, its concentration will vary with membrane concentration. The membrane pK, pK_m, is the pH in the aqueous phase at which the concentrations of the charged and uncharged species, in the membrane, are equal. pK_m does not depend on membrane concentration. The pK shift, ΔpK ($= pK_w - pK_m$, where pK_w is the pK in water), is a consequence of the different binding constants for the charged and uncharged species. This can be visualized in Scheme 1, where P^+ and P^0 are the partition coefficients for the charged and uncharged species, respectively.



Scheme 1.

pK_{app} and pK_m are related to experimentally determined partition coefficients [166]:

$$pK_{app} = pK_w + \log\{[(P^+ \cdot V_m) + V_w]/[(P^0 \cdot V_m) + V_w]\} \quad (3)$$

and

$$pK_m = pK_w + \log(P^+/P^0) \quad (4)$$

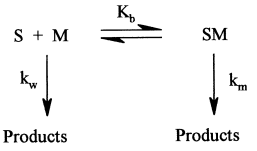
ΔpK can be positive, zero, or negative, depending on solute and membrane surface charge [166]. Garcia-Soto and Fernandez [169] have shown that, while ΔpK for TTC increases in the presence of negatively charged micelles, it decreases in the presence of non-ionic micelles, and decreases to a larger extent when the micelles are positively charged. This is due to the fact that in the first case $P_{charged} > P_{uncharged}$, while in the two latter systems the opposite is true. Similar results were found by Strichartz and coworkers [170], who determined pK shifts for a series of LA in micelles of variable surface charge.

pK shifts were found for a series of drugs upon binding to micelles or bilayers [104,158,165,168,171–174].

4.2. Effects of drug binding on reaction kinetics

The use of surfactants in pharmaceutical formulations is quite common not only as a tool to solubilize slightly soluble drugs but also to protect them from degradation. The stability of the drug in micelles may be altered favorably or unfavorably.

Oliveira and Chaimovich [175] have reviewed the effects of detergents and other amphiphiles on both the rate and the mechanism of reactions. The authors also provided a quantitative analysis of the micellar effects on the stability of β-lactam antibiotics in the presence of micelles [176,177] and emulsions [178],



Scheme 2.

emphasizing the effects of the aggregates on the rate and/or mechanism of the reaction.

Scheme 2 [179] presents the possible events for reactions occurring when a substrate partitions between water and a second phase.

Only a situation where there are no charge effects will be considered. The observed pseudo-first order rate constant, k_Ψ, is given by the equation:

$$k_\Psi = \frac{k_m[M] + k_w}{1 + k_b[M]} \quad (5)$$

where k_m and k_w are the pseudo-first order rate constants in the aggregate and in water, respectively, K_b is the binding constant, and [M] is the concentration of the aggregate-forming compound(s) [179].

k_m and k_w were determined for the alkaline hydrolysis of a spin-labeled benzoate [180] and of several TTC analogues in the presence of liposomes ([152], and Bianconi, Amaral and Schreier, in preparation). The determination of K_b at variable temperature yielded the thermodynamic parameters of binding: ΔG, ΔH, and ΔS. The data ($\Delta H > 0$, $\Delta S > 0$) showed that the binding was in agreement with the classical hydrophobic effect. Although ordering of the solute in the membrane environment should make a negative contribution, the loss of water of hydration must play a predominant role, determining the positive value of ΔS. Similar results were found for a homologous series of alcohols [181]. The TTC analogues were able to self-associate. In the aggregated form, the compounds were hydrolyzed at a slower rate [182].

Since aminoester anesthetics are cleared from the circulation upon hydrolysis by extracellular, non-specific esterases, these kinetic studies provide a rationale for the higher efficiency and toxicity of the more hydrophobic anesthetics [183].

Changes were observed in the kinetics and/or mechanism of reaction upon self-aggregation or incorporation into micelles, bilayers, or microemulsions of methylprednisolone 21-hemiesters [184], LA [185,186], carbaryl [187], 1,4-benzodiazepines [188], acetylsalicylic acid [189], and β-lactam antibiotics [190].

5. Phospholipidosis: a pathology induced by ionizable cationic amphiphilic drugs (CADs)

Phospholipidosis is a striking example of a pathological condition resulting from the interaction of amphiphilic drugs with membranes. Lipidosis-inducing CADs have a charged nitrogen group in close proximity to a hydrophobic moiety (an aromatic or aliphatic ring structure). Drugs belonging to different classes (antidepressant, anorexic, inhibitors of cholesterol synthesis, antihistaminic, antimalarial, antiinflammatory, antibacterial, antipsychotic, antiarrhythmic, antiestrogenic, and inhibitors of serotonin uptake) can induce lipidosis [191]. CADs cause phospholipid storage disorders; they are called lysosomotropic drugs because of their affinity for the acidic interior of lysosomes. It is believed that the uncharged form of the molecules crosses the lysosomal membrane to find a low pH milieu, favoring its rapid protonation. Phospholipids would bind to this form, taking it out of equilibrium; if enough drug enters the lysosome, the increase in pH could affect the activity of lysosomal enzymes [192].

Lipidosis is characterized by the appearance of lipid lamellar bodies or, less commonly, crystalloid inclusion bodies (identified with HII phase), inside the cell [192,193]. These structures originate from lysosomes. Their lipid composition is different from that in Niemann–Pick or Tay–Sachs diseases, and is characterized by an enhanced concentration of polar lipids. Acidic phospholipids, such as phosphatidylinositol and bis(monoacylglycerol)phosphate accumulate to a greater extent than zwitterionic phospholipids in hepatic and renal tissues [194,195], while PC increases mainly in the lung [196].

Theories for the occurrence of lipidosis include inhibition of lipid catabolism by drug–substrate complexes or by increased pH inside the lysosome, with inhibition of enzymes involved in phospholipid catabolism [195,197] and increased phospholipid biosynthesis [191,192,194]. It is also proposed that the drugs interfere with phospholipid metabolism by directly binding to enzymes. The inhibitory effect of

CADs upon phospholipid catabolizing enzymes was described for a series of drugs [195,198]. When the drug is rapidly metabolized in vivo, as is the case for several β-blockers [199] and LA, lipidosis does not occur, since the drugs are not accumulated inside the organelle.

Lipidosis can be reverted by stopping drug administration. Otherwise, the inclusion bodies appear to disintegrate with time into membranous and granular debris [192]. Pathological implications include corneal opacity [200], decreased glomerular filtration [201], acute renal failure [202], pulmonary pneumocystis [197] and fibrosis, increased hepatic density, and depressed immune response [191].

6. Solubilization and delivery of amphiphilic and hydrophobic drugs by synthetic and biological surfactants

Since many drugs are amphiphilic or hydrophobic, several problems arise with respect to their formulation, solubilization in body fluids, and interaction with barrier membranes in the organism, to reach their final targets. The effects of micellar solubilization on the solubility and absorption of non-polar solutes are quite well documented. A large number of systems, making use of micelles and other organized lipid assemblies, are presently under study for the purpose of drug delivery.

We will focus on work concerning the use of surfactants, in the form of micelles, as vehicles for sparingly water-soluble drugs. Solubilization is a consequence of the presence of hydrophobic domains in the surfactant aggregates acting as microenvironments for hydrophobic solubilizates. We will present studies of drug–surfactant interactions from a physicochemical standpoint, as well as applied aspects, such as drug bioavailability, absorption, and the multiple surfactant effects, including toxic ones. In some cases, solubilization has been found to be orders of magnitude larger than in the absence of surfactants.

Both synthetic and biological surfactants (mostly bile salts) have been employed. Although non-ionic surfactants are more physiologically tolerable than ionic ones, they usually have a lower solubilizing capacity. The polar region of many non-ionic surfactants consists of polyoxyethylene (POE) or polyethyleneglycol (PEG). Their micelles can be considered as formed by several microdomains: hydrocarbon, POE, POE/water. A cross section of a POE surfactant micelle thus offers a range of solubilization regions of different polarities.

6.1. Physicochemical aspects of solubilization

The thermodynamics of solubilization of a series of steroids (hydrocortisone, dexamethasone, testosterone, and progesterone) by long chain POE surfactants was investigated [203]. The partition coefficients increased with decreasing steroid polarity and decreased with temperature. The standard free energy change of solubilization, ΔG_s^0 decreased with decreasing steroid polarity, but was essentially temperature-independent. The change in enthalpy, ΔH_s^0 was negative indicating that solubilization was energetically favored. ΔH_s^0 became more positive as the steroid polarity decreased. Hydrocortisone and dexamethasone (more polar) were solubilized with a negative change in entropy, while testosterone and progesterone (less polar) provided a positive change. Two opposing factors were proposed to be involved: insertion of solubilized molecules in the micelles (a more ordered system) restricts the molecular motion and contributes to a decrease in entropy. On the other hand, when a relatively non-polar molecule migrates from the aqueous to the micellar phase, the loss of water of hydration causes a positive entropy change. For the more polar steroids, the negative contribution predominates, since, by being incorporated in the outer, more hydrated, POE shell, they very likely do not lose their hydration water.

The solubility of a series of barbituric acids increased with surfactant concentration and with temperature [204]. The apparent distribution coefficient decreased with temperature, indicating that the solubility in water increased faster than in the micelles. ΔG_s^0 and ΔH_s^0 were negative for the whole series of 13 barbituric acids, while ΔS_s^0 was negative, or zero, for nine of them. It was concluded that most of the drugs are absorbed in the micellar surface region. The enthalpy/entropy compensation phenomenon whereby ΔG_s^0 remains essentially constant with increasing temperature at the expense of compensatory changes in ΔH_s^0 and ΔS_s^0, was also discussed. Linear

correlations were found for enthalpy/entropy compensation, as well as between ΔG_s^0 and the molecular surface area of the compounds.

In various studies linear correlations were established between the micellar/water distribution coefficients and partition coefficients in octanol/water ($P_{o/w}$) for a series of poorly water-soluble drugs [205,206], pointing at the role of solute lipophilicity in solubilization.

A model was developed to estimate the increase of drug solubility as a function of micelle concentration [207]. An equation correlating the solubilization ratio ($SR = SC_{bs}/SC_{aq}$, where SC_{bs} and SC_{aq} are, respectively, the solubilization capacities in BS and in water) with log $P_{o/w}$ was established for a collection of steroids and found to apply, with minor modification, to a collection of non-steroidal, highly lipophilic compounds. The results indicated that the increase in drug solubility as a function of BS concentration can generally be predicted simply on the basis of $P_{o/w}$ and the compound's aqueous solubility.

When dealing with ionizable compounds, pH effects have to be taken into account. The solubilization of a series of substituted benzoic acids in non-ionic micelles was pH-dependent, increasing with decreasing pH, i.e. when the solutes became uncharged [208]. The partition coefficient of the unionized species increased with increasing lipophilicity of the benzoic acid derivative.

6.2. *Mechanisms for surfactant improvement of drug absorption: solubilization, wetting, and membrane effects*

The analysis of the mechanism of drug–surfactant interaction involves a complex ensemble of possibilities. Besides effective solubilization, phenomena like wetting play a role in drug dissolution. The mechanism of wetting implies that dissolution is favored by a reduction of the interfacial tension between the drug and the medium by the surfactant. Bakatselou and coworkers [209] studied the solubilization and wetting effects of a BS on the dissolution of steroids. For all compounds, wetting effects predominated over solubilization at low (pre-micellar) BS concentration, representative of the fasted state. For the more polar compounds, this trend continued at higher BS concentrations, typical of the fed state. Instead, for the more lipophilic compounds, solubilization was the predominant mechanism under the latter conditions. When comparing the dissolution of two non-steroidal antiinflammatory drugs by BS, it was found that, while solubilization was the prevailing mechanism for one drug, wetting was the dominant process for the other [210].

A large number of studies focus on the bioavailability and absorption of drugs in vivo when administered with BS. Hörter and Dressman [211] have reviewed the influence of physicochemical properties on the dissolution of drugs in the gastrointestinal (GI) tract. According to the authors, the rate-limiting step for drug absorption from the GI tract is often dissolution from the dosage form. Solubility in the GI contents is determined by aqueous solubility, crystalline form, drug lipophilicity, co-ingested foodstuffs, pK_a in relation to the GI pH profile, and solubilization by native surfactants. In the small intestine, micellar solubilization of the drug can occur when the amphiphilic bile components (BS, PC, and monoolein) reach concentrations higher than their cmc.

The results obtained in studies with BS or other largely employed surfactants are variable. BS reduced the bioavailability of the drug atenolol [212] and BS/oleic acid MM [213] did not have a pronounced beneficial effect on the transfer of lipophilic drugs in the rat small intestine. Nevertheless, it is remarked that in vivo these micelles can promote the overall process of absorption of poorly water-soluble drugs by enhancing their dissolution rate, which in many cases is the rate-limiting step [213]. Micelle solubilization of the antibiotic cefadroxil by sodium dodecyl sulfate (SDS), a surfactant widely used in drug formulations, was minimal [214]. The great increase in the drug's apparent absorption rate in the rat colon was ascribed to a direct effect of the surfactant on the absorbent membrane.

In vitro studies were conducted with gemfibrosil [215], a lipid-lowering agent, in model systems approximating the conditions of the upper GI tract, to identify drug physicochemical properties affected by endogenous and dietary lipids. When increasing amounts of bile components were added, the drug's solubility increased with respect to that in BS alone. Under conditions mimicking the fed state, the solu-

bility was improved when compared to the fasted state.

The fact that food intake can affect the bioavailability of poorly water-soluble drugs led to the investigation of the ability of freeze-dried drug milk formulations [216] and milk fat globule membrane [217] to bind several compounds and to influence their intestinal absorption. It was concluded that these systems have a potential for in vivo drug delivery.

That the surface activity of the drug itself can play a role in drug absorption was demonstrated by an increase in colonic permeability for the inhibitor of cholesterol biosynthesis, fluvastatin, with increasing drug concentration [218]. The effect was ascribed to a drug-promoted decrease of the surface tension at the membrane surface.

Nasal absorption is often employed for systemic delivery of protein and peptide drugs. Although small peptides can be readily absorbed through the nasal mucosa, larger molecules exhibit little or no bioavailability, and improvement in their absorption requires permeation enhancers. Among a number of surfactants, BS have been used to enhance the nasal or oral absorption, in particular of peptide drugs such as insulin [219], calcitonin [220], and growth hormone [221]. The data corroborated the hypothesis that BS act as absorption adjuvants by producing a high juxtamembrane concentration of insulin monomers via solubilization in MM, and by forming reverse micelles within nasal membranes through which the drug monomers can diffuse through polar channels from the nares to into the blood stream [219]. BS not only increased the oral absorption of human calcitonin, but also inhibited its degradation [220]. The pulsatile absorption kinetics observed after intranasal delivery of growth hormone [221] resembled its endogenous secretory pattern more closely than upon subcutaneous administration.

Several studies have focused on model and biological membrane-damaging effects by surfactants used in drug formulations [222–226]. Besides increasing drug solubility and dissolution rate, BS also increase absorption by altering the permeability of biological membranes. Indeed, elevated levels of BS and lysoPC in the stomach, as a result of bile reflux, have been implicated in gastric ulceration [227]. The compounds also caused the breakdown of mucus structure and were toxic to membranes. Low BS concentrations caused pronounced changes in the appearance of the mucosal surface [228] and accelerated the release of phospholipids and protein from the membrane [229]. A surfactant-induced increase in the permeability across various epithelia was suggested to be due to the concentration-dependent solubilization of cell membranes [230].

Although BS and lysoPC are apparently toxic to the gastric mucosa, as well as to in vitro preparations, no extensive destruction of the intestinal mucosa occurs. Normal human gall bladder bile contains about 85% water, 7.6% BS, 3% PC, 0.5% cholesterol and 3.5–4% other components [231]. Thus, a study was performed to verify whether PC, the major phospholipid in bile, would reduce the toxicity of BS and lysophospholipids to biological membranes by combining with them to form MM [222]. Indeed, PC incorporation into the aqueous medium reduced the toxicity caused by both BS and lysoPC. In addition, PC provided protection against membrane damage by SDS. MM formation was proposed to account for the protective effect and it was suggested that they could be convenient drug carriers.

6.3. *Mixed micelles as solubilizing agents*

MM have been extensively used as drug solubilizing agents, as well as delivery systems. Their toxicity or therapeutic index (TI) have been compared to those of other lipid carriers. Mixtures of sodium cholate and PC in water can achieve different organizational states depending on the proportion of the components: lamellar, cubical, hexagonal, micellar [231]. The extent of diazepam solubilization followed the order: lamellar > cubical > hexagonal > micellar. Phospholipid–detergent MM were also used to solubilize teniposide [232], taxol [233], hydrocortisone [234], gemfibrosil [215], retinoids [235], several benzodiazepines [236], and AmB (see below). MM were shown to convert to vesicles upon dilution, while keeping the water insoluble drug [232,233]. In most cases, MM were less toxic than single-component micelles of the corresponding surfactant. It was also seen that, while wetting effects predominate in the BS-only system, solubilization is the most important mechanism in MM for the dissolution of hydrocortisone. MM not only improved the solubility of

tetrazepam, but also increased the drug stability [237].

MM of variable composition have been investigated with respect to their ability to decrease AmB's toxicity and/or improve its TI.

The TI of AmB in phospholipid–BS MM was compared to that of the commonly used formulation Fungizone (AmB-DOC, 1:2) [238]. While K^+ leakage from RBC and cultured L cells treated with Fungizone was almost complete, MM had no effect. In contrast, both preparations were effective against fungal cells. Absorption and CD spectra showed that, while the antibiotic dissociated slowly in the monomeric form from the MM, it dissociated rapidly as a mixture of monomers and self-aggregates from Fungizone. The results corroborated the hypothesis that aggregated AmB is toxic to host cells, while the monomeric antibiotic can affect the viability of the infecting fungi. We have also shown that the toxicity of the antibiotic to RBC decreased, while its efficacy against fungal cells was retained when it was incorporated in a chylomicron-mimetic emulsion [239].

Dangi and coworkers [240] examined the solubility and GI membrane permeability of AmB in BS-containing MM and found that the drug's absorption rate was enhanced over 20-fold with respect to that of simple micellar systems.

MM of AmB and lauryl sucrose were examined, both with respect to their ability to bind to membrane sterols [241] and their activity and toxicity to fungal cells and RBC [242]. The data were compared with results found for AmB-DOC MM. In both cases, stoichiometries were found where AmB–surfactant complexes were formed, destroying the micellar structure. The interaction between AmB and lauryl sucrose was stronger than with DOC. It is also noteworthy that the DOC-AmB MM, where the antibiotic was in the monomeric form, was less toxic than Fungizone. At surfactant concentrations where AmB was monomeric, the preparations inhibited the toxicity to RBC and cultured mouse fibroblasts more than to fungal cells.

Based on the fact that heating AmB solutions leads to the formation of super-aggregates [59] Petit and coworkers [243] examined the effect of mild heating of Fungizone and of an AmB-DOC formulation on the in vivo therapeutic efficacy in experimental murine mycoses. An improvement of antifungal activity was obtained with the heated formulations due to their lower toxicity. It was suggested that mild heating of the already used pharmaceutical preparation could improve the drug's TI by reducing its toxicity to mammalian cells. Heat-induced super-aggregates were formed from AmB, Fungizone, and Amb-DOC preparations. It was observed that soluble aggregates, capable of interacting with cholesterol-containing membranes, were the toxic form of AmB.

Another colloidal delivery system, consisting of AmB and cholesteryl sulfate MM (ABCD, AmB colloidal dispersion, marketed as Amphocil) has been investigated [244]. A significant TI was found in animal studies, which was ascribed to stabilization of the MM by strong van der Waals forces. In this paper, a very interesting discussion about the conditions that favor the formation of large disk-like MM and about the concept of thermodynamic stability with regard to these and other colloidal systems is presented by the author.

AmB incorporated in polymeric micelles (PM) of a POE block poly(β-benzyl aspartate) copolymer (see below) yielded a very good loading efficiency, was non-hemolytic, and was released gradually, indicating that PM are a convenient system for AmB delivery [245].

The wealth of work on AmB shows that, although its DOC formulation (Fungizone) has been in clinical use for several decades, and some of the newer preparations are also starting to be used with patients, and in spite of the large amount of work on model systems, cells, and animal models, the basic physicochemical (thermodynamic and kinetic) rules underlying the aggregation behavior of this antibiotic are still unsolved.

6.4. PM as solubilizing agents

Long circulating carriers are desirable delivery systems in order to maintain blood levels of drugs, to achieve specific targeting (e.g. tumor cells), and for use with contrast agents (e.g. atherosclerosis diagnosis) [246]. Liposomes and microspheres are quite readily scavenged in a non-specific manner by the reticuloendothelial system (RES) [247].

PM consist of A-B or A-B-A block copolymers, where A represents hydrophilic and B hydrophobic

polymeric chains. Such micelles provide increased water interaction, decreased retention by RES, and prolonged circulation time in the blood [246,248,249]. Usually, the hydrophilic portion of PM consists of POE. POE is a low toxicity biomedical polymer and, when present at interfaces or surfaces, has the ability to suppress cellular and protein adsorption, therefore, it does not bind to blood components. PM circulate in blood for long times by escaping the renal filtration due to their hydrated outer shell and larger size, relatively to the isolated polymer chains [247]. In low vascular permeability tumors, carriers with a smaller size than liposomes may provide more efficient drug delivery [250].

Pluronic polyols are block copolymers of hydrophobic polyoxypropylene (POP) and hydrophilic POE; their micelles (diameter ca. 50 Å) can be effectively placed in the colloidal size range [249]. They are among the less toxic surface active agents and are widely used for drug delivery [251–253]. In other PM, biodegradable compounds such as polypeptide chains, mainly of Asp [246,254], benzyl-Asp [255] or Ala [256] form the core of the particles. Also polylactic acid [257] and polyglycolic acid [258] have been used.

Chung et al. [259] reported the use of PEG-*N*-isopropylacrylamide, a thermo-responsive (intelligent) block copolymer, expected to induce selective accumulation of anticancer drugs controlled by temperature modulation. Moreover, block copolymers have extended the use of non-ionic detergents into a new domain with regard to membrane disrupting activity. By controlling of the chemical structure of block copolymers, tunable membrane-disrupting agents have been synthesized [260].

7. Hydrotropy

Another approach to improve the solubility of sparingly water-soluble drugs consists in using the concept of hydrotropy. Hydrotropes are molecules that, at reasonably high concentrations, promote solubilization. Among well-known hydrotropic agents are urea, caffeine and other xanthine derivatives, tryptophan, certain antihistamines, sodium benzoate, sodium salicylate, and nicotinamide [261].

Several mechanisms have been proposed to account for the molecular process of solubilization: π–π complex formation, salting-in, changes in the nature of the solvent and hydrotrope aggregation [261]. It is believed that the mechanism varies for different systems.

The increase of the aqueous solubility of the cytotoxic agent chartreusin in the presence of hydroxybenzoates was interpreted as resulting from a plane-to-plane orientation of the drug and hydrotrope molecules brought together by electrostatic and hydrophobic interaction; solubilization was interpreted in terms of micellization [262].

Coffman and Kildsig examined the solubilization of riboflavin by nicotinamide and proposed that hydrotrope self-association plays a role in the mechanism of solubilization [261]. In contrast, complex formation was proposed to be the mechanism for the solubility enhancement of anticancer nucleoside analogues [263] and of a bisnaphthalimide tumoricidal agent, DMP 840 [264], by nicotinamide. Improved solubilization of rhodium carboxylate adducts, a new class of sparingly soluble cytotoxic compounds, was achieved by complexation with isonicotinic acid [265]. Complexation was also invoked as the mechanism of solubility enhancement of thiocetazone, a drug used in the treatment of tuberculosis, by isoniazid [266].

The behavior of hydrotropes, lower alkanoates, alkyl sulfates and alkylbenzene sulfonates, was compared to that of their longer analogues, known to form micelles [267]. The hydrotropes were found to self-associate and form non-covalent assemblies of lowered polarity beyond a certain concentration (minimal hydrotrope concentration). The aggregates were found to consist of planar or open-layer structures, instead of more compact, spherical micelles. In this case, solubilization would occur in the aggregate lipophilic region.

8. Prodrugs

In order to improve the solubility of a poorly soluble drug, prodrugs, either water-soluble or organized in a micellar arrangement, can be synthesized and converted to the active parent compound in vivo. Micellar prodrugs have additional advantages in that their micelles solubilize poorly soluble degrada-

tion products which can otherwise precipitate, and may act as self-stabilizing due to protection of the hydrolytically labile prodrug linkage within the micelle interior [268].

Some hemiester derivatives of water insoluble corticosteroids self-associate in water to form micelles. This approach was used in the development of micellar prodrugs of methylprednisolone [268]. Cholesteryl ester prodrugs of ibuprofen and flufenamate were synthesized and solubilized in microemulsions [269]. Self-association of a prodrug of phenytoin also caused a decrease in its rate of hydrolysis, as well as increased solubilization of the parent drug [270]. Similarly, increased solubilization was achieved by synthesizing PEG-derived prodrugs of amino group-containing compounds leading to extended plasma circulation half-lives and, in the case of anticancer agents, apparent tumor accumulation [271].

9. Surfactants as drugs

In view of their ability to alter the permeability of cells, surfactants display antibacterial properties by acting on bacterial cell walls. Long-chain surfactants containing quaternary ammonium or pyridinium ions as headgroups have been used as bactericidal or bacteriostatic agents. Kopecky [272] has reviewed this topic. More recently, vesicles made of a double-chain quaternary ammonium ion were also shown to have bactericidal properties [273].

Trimethyl alkylammonium compounds, with alkyl chains ranging from two to 16 carbons, display ultra-long lasting anesthetic activity [274] that increases with increasing chain length. The C12 derivative produced almost irreversible (17–20 days) anesthesia. Neurotoxic effects render these compounds unacceptable for clinical use; the authors ascribed these effects to the detergent nature of the compounds.

10. Concluding remarks

The data presented in this work have focused on surface active drugs. Studies investigating their self-association properties were described. As for their effects upon membranes, the literature presented demonstrates that, whatever the detailed mechanism of their membrane activity and whatever the nature of their self-association, amphiphilic drugs interact with membranes, exerting a variety of effects, at the molecular level, from changes in lipid organization to channel formation, induction of lipid flip-flop and of non-bilayer and interdigitated phases. These effects correlate with cell shape changes, membrane vesiculation, disruption, and, finally, solubilization. The effects of drugs are analogous to those of classical detergents. Similarly to the latter, drugs are able to extract membrane lipids and proteins. Pathological consequences of the interaction between cationic amphiphilic drugs and membranes were discussed.

It was shown that physicochemical properties (degree of ionization, reaction kinetics) are modulated by the drugs self-association or by their binding to other lipophilic aggregates.

Another important issue concerning amphiphilic and hydrophobic drugs is their solubilization. Solubilization by classical surfactants, mixed micelles, polymeric micelles, and hydrotropes was discussed from the physicochemical point of view and in terms of their use as drug carriers and absorption in the organism. The use of micellar prodrugs and of classical surfactants as drugs was also reported.

Advances in the understanding of the molecular mechanisms of amphiphiles self-aggregation, of their interaction with membranes, and of their solubilization by different surfactants will allow the rational design of more effective and less toxic therapeutic agents and delivery systems.

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